

AMENDMENT TO CLAIMS

1. *(Currently Amended)* A method for the preparation of a polypeptide of interest in authentic form, said method comprising the steps of:
 - (i) providing a fusion protein comprising, from its N-terminal to its C-terminal, (a) a fusion partner, (b) a Granzyme B protease recognition site comprising a Granzyme B protease cleavage site, (c) [[a]] the polypeptide of interest, wherein said cleavage site is adjacent to the polypeptide of interest, and
 - (ii) contacting said fusion protein with Granzyme B protease to cleave it at said cleavage site to yield said polypeptide of interest in authentic form.
2. *(Currently Amended)* [[A]] The method according to claim 1, wherein the Granzyme B protease recognition site comprises an amino acid sequence of the general formula:
P4 P3 P2 P1 ↓ (SEQ ID NO: 59)
wherein
P4 is amino acid I or V,
P3 is amino acid E, Q or M,
P2 is X, where X denotes any amino acid,
P1 is amino acid D, and
↓ is said Granzyme B protease cleavage site.
3. *(Currently Amended)* [[A]] The method according to claim 1, wherein the Granzyme B protease recognition site has an amino acid sequence selected from the group consisting of ICPD↓ (SEQ ID NO: 61), IEAD↓ (SEQ ID NO: 62), IEPD↓ (SEQ ID NO: 63), IETD↓ (SEQ ID NO: 64), IQAD↓ (SEQ ID NO: 65), ISAD↓ (SEQ ID NO: 66), ISSD↓ (SEQ ID NO: 67), ITPD↓ (SEQ ID NO: 68), VAPD↓ (SEQ ID NO: 69), VATD↓ (SEQ ID NO: 70), VCTD↓ (SEQ ID NO: 71),

VDPD↓(SEQ ID NO: 72), VDSD↓(SEQ ID NO: 73), VEKD↓(SEQ ID NO: 74), VEQD↓(SEQ ID NO: 75), VGPD↓(SEQ ID NO: 76), VEID↓(SEQ ID NO: 77), VRPD↓(SEQ ID NO: 78), VTPD↓(SEQ ID NO: 79), LEED↓(SEQ ID NO: 80), LEID↓(SEQ ID NO: 81), LGND↓(SEQ ID NO: 82), LGPD↓(SEQ ID NO: 83), and AQP↓(SEQ ID NO: 84), and wherein ↓ is said Granzyme B protease cleavage site.

4. *(Currently Amended)* [[A]] The method according to claim 2, wherein the general formula furthermore comprises the amino acids P1' and P2' resulting in the general formula P4 P3 P2 P1↓P1'P2' (SEQ ID NO: 60), wherein P1' is X where X denotes any amino acid, P2' is G, and wherein P1' and P2' is a part of the polypeptide of interest.
5. *(Currently Amended)* [[A]] The method according to claim 2, wherein the general formula furthermore comprises the amino acids P1', P2', P3' and P4' resulting in the general formula P4 P3 P2 P1↓P1'P2'P3'P4' (SEQ ID NO: 93), wherein P4' is D or E, and wherein P1', P2', P3' and P4' is a part of the polypeptide of interest.
6. *(Currently Amended)* [[A]] The method according to claim 1, wherein the polypeptide of interest is selected from the group consisting of an enzyme, a polypeptide hormone, a single chain antibody variable region fragment, and apolipoprotein A.
7. *(Currently Amended)* [[A]] The method according to claim 6, wherein the polypeptide hormone is selected from the group consisting of somatotrophin, glucagon, insulin and interferon.
8. *(Currently Amended)* [[A]] The method according to claim 6, wherein the enzyme is Granzyme B.

9. *(Currently Amended)* [[A]] The method according to claim 1, wherein the fusion partner is an affinity-tag.
10. *(Currently Amended)* [[A]] The method according to claim 9, wherein the affinity-tag is selected from the group consisting of a polyhistidine-tag, a polyarginine-tag, a FLAG-tag, a Strep-tag, a c-myc-tag, a S-tag, a calmodulin-binding peptide, a cellulose-binding peptide, a chitin-binding domain, a glutathione S-transferase-tag, and a maltose binding protein.
11. *(Currently Amended)* [[A]] The method according to claim 1, wherein the Granzyme B protease is selected from the group consisting of human Granzyme B protease, mouse Granzyme B protease and rat Granzyme B protease.
12. *(Withdrawn)* A method according to claim 11, wherein the Granzyme B protease is a human Granzyme B protease variant as shown in SEQ ID NO 57, wherein the Cystein residue no. 228 (chymotrypsinogen numbering) is mutated to Phenylalanine.
13. *(Currently Amended)* [[A]] The method according to claim 1, wherein the Granzyme B protease is in an immobilised form.
14. *(Currently Amended)* [[A]] The method according to claim 13, wherein the Granzyme B protease is immobilised via the C-terminus.
15. *(Currently Amended)* [[A]] The method according to claim 13, wherein the Granzyme B protease is immobilised via a lysine amino acid residue.
16. *(Currently Amended)* [[A]] The method according to claim 10, wherein the affinity-tag is a polyhistidine-tag, and wherein the fusion protein is contacted with said Granzyme B protease in the presence of Ni^{2+} ions and Nitrilotriacetic Acid (NTA).

17. *(Currently Amended)* [[A]] The method according to claim 16 [[15]], wherein the concentration of Ni^{2+} is in the range of 1-20 mM, and the concentration of NTA is in the range of 1-20 mM.
18. *(Withdrawn)* A fusion protein comprising, from its N-terminal to its C-terminal, (a) a fusion partner, (b) a Granzyme B protease recognition site comprising a Granzyme B protease cleavage site, and (c) a polypeptide of interest, wherein said cleavage site is adjacent to the polypeptide of interest.
19. *(Withdrawn)* A fusion protein according to claim 18, wherein the Granzyme B protease recognition site comprises an amino acid sequence of the general formula:
P4 P3 P2 P1 ↓
wherein
P4 is amino acid I or V,
P3 is amino acid E, Q or M,
P2 is X, where X denotes any amino acid,
P1 is amino acid D, and
↓ is said Granzyme B protease cleavage site.
20. *(Withdrawn)* A fusion protein according to claim 18, wherein the Granzyme B protease recognition site has an amino acid sequence selected from the group consisting of ICPD↓, IEAD↓, IEPD↓, IETD↓, IQAD↓, ISAD↓, ISSD↓, ITPD↓, VAPD↓, VATD↓, VCTD↓, VDPD↓, VDSD↓, VEKD↓, VEQD↓, VGPD↓, VEID↓, VRPD↓, VTPD↓, LEED↓, LEID↓, LGND↓, LGPD↓, and AQPD↓, and wherein ↓ is said Granzyme B protease cleavage site.
21. *(Withdrawn)* A fusion protein according to claim 19, wherein the general formula furthermore comprises the amino acids P1' and P2' resulting in the general

formula $P4\ P3\ P2\ P1\downarrow P1'P2'$, wherein $P1'$ is X where X denotes any amino acid, $P2'$ is G, and wherein $P1'$ and $P2'$ is a part of the polypeptide of interest.

22. *(Withdrawn)* A fusion protein according to claim 19, wherein the general formula furthermore comprises the amino acids $P1'$, $P2'$, $P3'$ and $P4'$ resulting in the general formula $P4\ P3\ P2\ P1\downarrow P1'P2'P3'P4'$, wherein $P4'$ is D or E, and wherein $P1'$, $P2'$, $P3'$ and $P4'$ is a part of the polypeptide of interest.
23. *(Withdrawn)* A fusion protein according to claim 18, wherein the polypeptide of interest is selected from the group consisting of an enzyme, a polypeptide hormone, a single chain antibody variable region fragment, and apolipoprotein A.
24. *(Withdrawn)* A fusion protein according to claim 23, wherein the polypeptide hormone is selected from the group consisting of somatotrophin, glucagon, insulin and inteferon.
25. *(Withdrawn)* A fusion protein according to claim 23, wherein the enzyme is Granzyme B.
26. *(Withdrawn)* A fusion protein according to claim 25, wherein Granzyme B comprises a C-terminal polyhistidine-tag.
27. *(Withdrawn)* A fusion protein according to claim 25, selected from the group consisting of pro-IEPD-GrB-H6 (SEQ ID NO 2) and pro-IEAD-GrB-H6 (SEQ ID NO 3).
28. *(Withdrawn)* A fusion protein according to claim 25, selected from the group consisting of pro-IEPD-GrB-H6 C228A (SEQ ID NO 5), pro-IEPD-GrB-H6 C228T (SEQ ID NO 6), pro-IEPD-GrB-H6 C228V (SEQ ID NO 7), and pro-IEPD-GrB-H6 C228F (SEQ ID NO 8).

29. *(Withdrawn)* A fusion protein according to claim 25, wherein the enzyme Granzyme B is a human Granzyme B protease variant wherein the Cystein residue no. 228 (chymotrypsinogen numbering) is mutated to Phenylalanine.
30. *(Withdrawn)* A fusion protein according to claim 25, wherein the human Granzyme B protease variant is as shown in SEQ ID NO 57.
31. *(Withdrawn)* A fusion protein according to claim 18, wherein the fusion partner is an affinity-tag.
32. *(Withdrawn)* A fusion protein according to claim 31, wherein the affinity-tag is selected from the group consisting of a polyhistidine-tag, a polyarginine-tag, a FLAG-tag, a Strep-tag, a c-myc-tag, a S-tag, a calmodulin-binding peptide, a cellulose-binding peptide, a chitin-binding domain, a glutathione S-transferase-tag, and a maltose binding protein.
33. *(Withdrawn)* A human Granzyme B protease variant wherein the Cystein residue no. 228 (chymotrypsinogen numbering) is mutated to Phenylalanine.
34. *(Withdrawn)* A human Granzyme B protease variant according to claim 33, as shown in SEQ ID NO 57.
35. *(Withdrawn)* A method of cleaving a fusion protein comprising contacting said fusion protein with the human Granzyme B protease variant according to claim 33.
36. *(Withdrawn)* An isolated nucleic acid sequence encoding the fusion protein according to claim 19 or the human Granzyme B protease variant according to claim 33.

37. *(Withdrawn)* A recombinant vector comprising the isolated nucleic acid sequence according to claim 36.
38. *(Withdrawn)* A host cell transformed with a vector according to claim 37.
39. *(Withdrawn)* A method for the production of a fusion protein according to claim 18 or a human Granzyme B protease variant according to claim 33, comprising the steps of:
- (i) providing a recombinant vector comprising the isolated nucleic acid sequence according to claim 36 operatively linked to a promotor,
 - (ii) transforming a host cell with said recombinant vector,
 - (iii) culturing said host cell under conditions to express said fusion protein or human Granzyme B protease variant, and
 - (iv) optionally isolating said fusion protein or human Granzyme B protease variant.